C·R·E·S

Center For Reproduction Of Endangered Species

1990 Summer Report

Editor: Judy Kinsell

• DIRECTOR'S MESSAGE

The extinction of animal species has accelerated to the point where one species of mammals and one of birds pass into oblivion each year. If we add the other animals—insects, fish, amphibians, reptiles and the rest, the rate of extinction is one per day. And, if we continue at the present rate of habitat destruction, we could lose one bird or mammal species per day by the year 2000.

People are crowding wild animals out of existence. Wild habitat is being destroyed at an unbelievable rate as more and more people demand more and more resources. What we are attempting to do with CRES is hedge against the economic, social and political forces which so threaten wildlife and yet seem to be beyond our immediate control. The longer we keep endangered species alive and reproducing, in captivity and in the wild, the greater the chance our grandchildren will share fully in the wonders of nature.

CRES uses a unique multidisciplinary approach in its effort to rescue animals headed for extinction. Our behaviorists study the influence of the environment on reproductive potential, and endocrinologists monitor and evaluate hormone levels which stimulate and regulate the function of reproductive organs. In the Genetics Division, genetically sound breeding strategies for endangered species are charted.

In the areas of virology and immunology, CRES scientists work to reduce the impact of deadly diseases by identifying causes and improving preventive measures. The Reproductive Physiology Division conducts studies in the areas of semen evaluation, cryopreservation, artificial insemination and embryo transfer. Pathology studies often contribute to the survival of species by pinpointing the cause of death.

We at CRES are personally committed to carrying out the highest quality work, maintaining our strong foundation in basic knowledge, and using the most current techniques in our respective fields of expertise. We are grateful for the support CRES has received from corporations, foundations and individuals who believe in our mission. Together we can continue the growth and productivity which has made CRES an internationally recognized center of excellence in zoo research.

—Dr. Werner Heuschele

• REPRODUCTIVE PHYSIOLOGY

CRES Reproductive Physiologist Dr. Barbara Durrant and her team are working to perfect artificial insemination techniques to enhance reproduction of the rare and extraordinarily beautiful Chinese monal. The focus of the investigation is to find a way to freeze and thaw monal semen without losing its viability, or motility.

"Typically, bird semen is about 75 percent motile when it goes into the freezer, and only 20 or 30 percent motile when it comes back out," said Dr. Durrant. "We could get around that by inseminating females with three or four samples, but the volume would be too great. What we need to do is reduce the volume of sperm and increase the population of motile sperm. The only way to do that is to eliminate the nonmotile sperm."

As in human medicine, the researchers use a centrifuge with an added gradient to spin and separate motile from nonmotile semen. A cryoprotectant coating used in freezing the sperm must also be removed after thawing, or it will interfere with the female's fertility when she is inseminated. As the semen is pulled through the gradient in the centrifuge, it should strip off the cryoprotectant. By modifying the procedure, Dr. Durrant found that using a very mild centrifugation and a low density gradient called percol, brought encouraging results. The sperm appeared to be healthy and motile without evidence of freezing injury.

Because the Chinese monal is severely endangered, the CRES team is conducting the study with the Himalayan monal, a closely related species. Says Dr. Durrant, "We're assuming that these species are so close that methods we develop for the Himalayan monal will be applicable." Himalayan monals were artificially inseminated with frozen semen using the percol gradient technique, and at this writing, two fertile eggs have been produced.

The San Diego Zoo's collection includes a pair of Chinese monals and one male offspring, the only representatives of their species exhibited outside of China. We have had some success in breeding these rare pheasants, but we need to increase their genetic variability. By finding a way to freeze and thaw monal semen while preserving its motility, we may one day be able to bring samples from China for use in artificial insemination, and to freeze for the future.

• ENDOCRINOLOGY

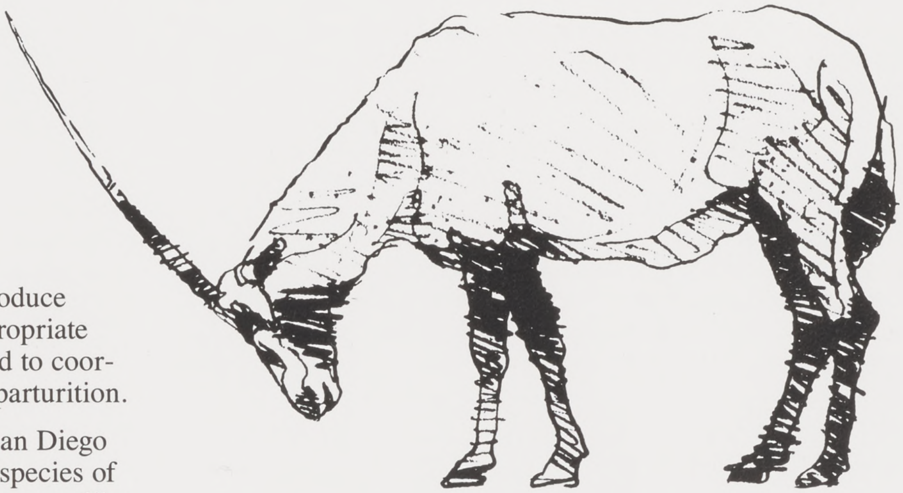
One of the first urinary hormone assays established at CRES was designed to chart estrus cycles and determine pregnancy among ruffed lemurs. The assay was an effective technique, so researchers relied on the lemur's hormone profile when attempting to create an assay for the slow loris, a relative of the lemur.

But progress in setting up an assay for the loris was as slow as the animal itself. Three years into an investigation, Lorelei Perez, a student from the University of California–San Diego, and volunteer at CRES, was unable to establish a hormone profile for the slow loris. She went to Duke University to take urine samples from their breeding group, but still the technique refused to work. Finally, under the direction of CRES endocrinology specialist Nancy Czekala-Gruber, Ms. Perez performed a metabolism study, whereby a small amount of hormone is injected into the system and tracked to see how it is processed by the body.

The results of the metabolism study were enlightening–instead of finding hormones in the urine, the majority were discovered in the feces. The study also revealed the form that the hormone assumed since it had been metabolized by the body. The information gained through this test led to an effective estrus and pregnancy detection technique, which is in use today. Said Ms. Czekala-Gruber, "Now we can determine when a female is beginning to cycle, and we are able to diagnose pregnancy 40 days after breeding. Forty days is quite a long time compared to some of our other techniques, but it's still a very competent type of system."

The CRES hormone assay also enables behaviorists to manage the off-exhibit loris collection more effectively

For information on how you can contribute to CRES, please call Amy Parrott at (619) 231-1515, extension 4249.



for optimum breeding. It allows them to introduce normally aggressive males to females at appropriate times; to pinpoint the date of conception; and to coordinate behavioral observation in advance of parturition.

With 10 slow lorises and 12 pygmy lorises, San Diego has the largest combined collection of these species of any zoo in the country. Successful breeding groups like San Diego's are becoming increasingly valuable as the loris' native rain forest habitat in Southeast Asia diminishes. Captive propagation may well be the only way to ensure survival of these fascinating primitive primates.

• GENETICS

The Arabian oryx is one of the rarest ungulates in the world. Because people once believed that killing them would impart great strength, they were slaughtered to extinction in the wild by the early 1970s. Today, small herds of Arabian oryx bred in captivity at the San Diego Wild Animal Park and other zoos are being reintroduced into their native desert in central Oman. As the oryx population begins to regenerate, scientists are concerned with maintaining the genetic integrity of the species.

A recent study conducted by French scientists at Taif, Saudi Arabia, revealed that, of 62 Arabian oryx surveyed, seven had a different karyotype, or chromosome configuration. Rather than the normal 58 chromosomes, six oryx had 57 and one had only 56. The French surmised that this polymorphism, involving a fusion of chromosomes, was a natural occurrence in the Arabian oryx population.

CRES geneticist Dr. Oliver Ryder and cytogeneticist Arlene Kumamoto, with researcher Steve Kingswood, reviewed data they had collected involving captive-bred Arabian and scimitar-horned oryx, and found evidence of polymorphism in the latter only. The CRES findings suggested that the French may have witnessed a hybridization between the two species, rather than a naturally occurring phenomenon.

The possibility of hybridization in the Arabian oryx population was of great concern to the Omanis, as their animals are the only free-ranging Arabian oryx in the world. Hybridization not only would have compromised the integrity of the oryx as a biological species, but could result in reduced fertility in the herd. Some of the animals in the reintroduction program had come from Thumama, Saudi Arabia, where Arabian/scimitar-horned oryx hybrids were known to exist. The Advisor for Conservation of the Environment to the Sultanate of Oman contacted Dr. Ryder and Ms. Kumamoto requesting chromosome evaluations on some of their animals.

Said Ms. Kumamoto, "We found that two free-ranging male oryx had 57 chromosomes, while three captive animals in Muscat had 58. Through a process called G-banding analysis we determined that the polymorphism found in the Arabian oryx is different from that of the scimitar-horned oryx polymorphism. The fusion chromosomes in the two species were different, and indicated that the polymorphism in the Arabian oryx was not due to hybridization with the scimitar-horned oryx."

• PATHOLOGY

The California Condor Recovery Program has made enormous strides since its inception in 1975, but there have been challenges along the way. The most recent of these was the first death at the San Diego Wild Animal Park of a captive-bred California condor embryo. Pathologist Dr. Marilyn Anderson performed the post-mortem examination of the chick, which died in the pip stage just prior to hatching.

"The chick's death was associated with the accumulation of excess fluid beneath the skin, internal membranes, and in the lungs due to a thicker than normal egg shell," explained Dr. Anderson. "The egg shell thickness probably contributed to inadequate fluid loss that normally occurs during incubation. In addition, microscopic changes indicated kidney failure, a complication stemming from an excessive amount of fluid in cells and tissues."

Understanding why an individual animal has died can be crucial in deciding whether changes are needed in animal husbandry and health care. At this writing, the parents of the dead condor chick have renewed their courtship, and may produce a second fertile egg. Because thick-shelled eggs are typical of this mother, the California Condor Recovery Team is reviewing options for incubation modifications to help assure survival of the second chick. Data gathered from the pathology studies are vital to this process.

The Pathology Division has made significant contributions toward the success of the California Condor Recovery Program. Dr. Anderson's previous work with the condor includes necropsies of wild condors that died from 1980 to 1986. Her findings provided important support for the decision to bring the few remaining birds into a protected environment for safe, captive propagation.

• **ACHIEVEMENTS** CRES comparative physiologists, Drs. John Phillips and Nancy Pratt, were recently awarded a grant by the National Science Foundation to study the savanna monitor lizard. The scientists are conducting field studies in Africa and plan to complete their laboratory work in San Diego. The investigation will provide information on the lizard's hormone/behavior interactions and environmental requirements for successful egg incubation.

All species of monitor lizards are listed as threatened or endangered, so the savanna monitor will serve as a model from which the scientists can learn more about all monitors, including the giant Komodo dragon. The grant of $49,219 was one of only four awarded nationwide out of some 200 applicants.

CRES behaviorist Dr. Donald Lindburg is the recipient of the Centennial Award for Excellence in Zoo Research, granted by the National Zoological Park in Washington, D.C. Dr. Lindburg was honored for his paper, "Proceptive Calling by Female Lion-Tailed Macaques," which explores his discovery that females of the species solicit sexual contact with males outside their family groups by using a unique call. The lion-tailed macaques' calls indicate the females are not just passive participants in courtship rituals. That may challenge some long-held notions about primate breeding behaviors.

Dr. Lindburg's work was judged best in a nationwide competition to promote and reward excellence in zoo research. The National Zoological Park and the Friends of the National Zoo sponsored the competition in conjunction with the National Zoo's 100th anniversary celebration.

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